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## CRITICAL REVIEW OF TISSUE-BASED SELENIUM TOXICITY THRESHOLDS FOR FISH AND BIRDS

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### ABSTRACT

Selenium-induced fish kills in Belews Lake, North Carolina in the late-1970s resulted in a substantial amount of research on selenium effects to aquatic life. Similarly, selenium-induced teratogenesis in aquatic birds at Kesterson Reservoir, California in the mid-1980s resulted in extensive research on selenium effects to aquatic birds. Unlike many other contaminants for which water exposure is the critical pathway for environmental effects, selenium ecotoxicology is driven by bioaccumulation in invertebrates and exposure to fish and birds via the diet. At sufficiently high levels, these exposures result in embryo teratogenesis and reduced survival of larval fish and bird chicks.

Because of the dietary exposure pathway, assessment of selenium risks to the environment is best accomplished by evaluating concentrations in fish and bird tissues rather than the water. One of the end results of the intensive research efforts mentioned above has been the publication of tissue-based toxicity thresholds for both fish and birds by the U.S. Fish and Wildlife Service (USFWS). These thresholds are being used in the United States and Canada for environmental assessments and for establishing clean-up guidelines for remediation/reclamation projects.

We critically evaluated these thresholds and the scientific literature upon which they are based. Specifically, we evaluated the USFWS proposed thresholds for selenium in fish whole body tissue (4 mg/kg dw), fish ovaries (10 mg/kg dw), fish diets (3 mg/kg dw), and bird eggs (6 mg/kg dw). We observed that the USFWS thresholds for fish tissues appear overly conservative and do not appear to be well supported by the scientific literature. In several cases, the USFWS interpretation of the studies on which the thresholds are based are contrary to our interpretation, that of U.S. EPA, and the authors that published the study. Similarly, for birds we found that the USFWS threshold is conservative, does not recognize differences in site-specific bioaccumulation and is based on field studies using an endpoint not specific to selenium, chick mortality. Because the details of the study and raw data are not readily available, it is difficult to ascertain whether the study adequately controlled for confounding factors such as disease, predators, weather and other contaminants. This concern is heightened by the fact that the field data does not agree with results from controlled laboratory studies evaluating the same endpoint.

Considering the above, we re-examined all of the existing data and established alternative thresholds that we believe are better supported by the scientific evidence available. Specifically, we proposed thresholds for fish whole body, ovary, and diet of 6-9, 17, and 10-11 mg/kg dw and a bird egg threshold of 16 mg/kg dw. The details of our analyses and justification for the alternative thresholds are presented in this paper.

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## INTRODUCTION

As a result of events at Belews Lake, North Carolina and Kesterson Reservoir, California, the environmental toxicology of selenium has been intensely studied over the past 25 years in both the laboratory and field. The primary effect of selenium on aquatic life and birds is teratogenesis of developing embryos. At high concentrations, this effect can manifest as gross deformities and abnormalities in bird and fish embryos. At lower concentrations, teratogenesis is much more difficult to observe, but systemic abnormalities normally result in chick or larval fish mortality within several weeks of hatch (DeForest *et al.*, 1999; Fairbrother *et al.*, 1999).

Unlike many chemicals in aquatic systems, the dietary exposure pathway is of far more importance than the water exposure pathway. Inorganic selenium forms (e.g., selenate, selenite) have relatively limited bioavailability compared with organo-selenium forms. Typically, inorganic selenium released to aquatic systems is reduced and biotransformed to organo-selenium compounds (Bowie *et al.*, 1996). These compounds are then bioaccumulated by organisms at the base of the food chain and subsequently accumulated by organisms higher in the food chain that prey on them (Ogle and Knight, 1996). Selenium effects on invertebrates typically occur at concentrations much higher than those that elicit effects on the vertebrates (e.g., fish and birds) that prey upon them. Consequently, fish and birds are the most sensitive groups to selenium poisoning and the focal point of most environmental assessments (Ogle and Knight, 1996; Skorupa *et al.*, 1996).

The concentrations that occur in prey or predator tissues are highly site-specific in nature, being influenced by selenium concentrations, water quality conditions that influence selenium reduction (redox, pH), biological productivity which influences the rate of biotransformation, and the food chain pathways that lead to sensitive vertebrate predators. As a result, evaluating potential selenium impacts at a site is best accomplished by measuring selenium concentrations in tissues rather than in water (Adams *et al.*, 1998; Skorupa *et al.*, 1996). This then necessitates having reliable tissue-based toxicity thresholds against which the tissue data can be assessed.

An important outcome of the field and laboratory studies over the past 25 years has been the development of tissue-based toxicity thresholds. Studies have been conducted by a wide variety of scientists, but the U.S. Fish and Wildlife Service (USFWS) has taken a leading role in synthesizing these data and developing toxicity thresholds. Over the past ten years, USFWS has published a number of toxicity thresholds for fish and birds (Lemly, 1996; Skorupa, 1998). The purpose of our study was to critically

evaluate the scientific justification for the thresholds they developed and where appropriate, develop alternative thresholds that we believe are better supported by the existing data.

## **METHODS AND MATERIALS**

Our study involved summarizing all of the available data used by USFWS in developing their thresholds and identifying any additional data appropriate for use in deriving thresholds that may have been missed by USFWS or published since their thresholds were developed. We evaluated the data for fish and birds separately. Consistent with USFWS, for fish we evaluated a variety of tissues (whole body, ovary, diet) while for birds we focused on eggs. Eggs or ovaries are the ideal tissue for assessment because it is the site of toxic action for selenium. However, sampling ovaries in fish can be difficult for small species, necessitating consideration of other tissue types.

### **Toxicological Data for Fish**

A total of 24 studies were identified in our review of the literature. Most of these studies included a dietary component to the exposure regime, those that did not were excluded from further analysis. Initial data analysis indicated there may be differences in sensitivity between warmwater fish and coldwater anadromous salmonids, and so the data were divided accordingly for further analysis. To evaluate the studies further, we fit statistical models (probit, logit) to the toxicity data for the various tissues and endpoints. We estimated EC10, EC20, and EC50 values for the different tissues. Where insufficient data were available from a single study to derive a dose-response relationship, we pooled data from different studies that used similar endpoints and species (e.g., warmwater versus. coldwater). For consistency, all tissue concentrations are expressed on a dry weight (dw) basis. When tissue concentrations were reported on a wet weight basis in the original study, we assumed a moisture content of 75% for all tissues except ovaries, for which we assumed an 85% moisture content (Gillespie and Baumann, 1986; Lemly, 1993). A detailed summary of these studies can be found in DeForest *et al.* (1999).

## **Toxicological Data for Birds**

Compared with fish, the total number of studies and diversity of bird species that have been studied is significantly less. Summaries of available data can be found in Fairbrother *et al.* (1999) and Skorupa *et al.* (1996). Two primary endpoints have been evaluated for birds, teratogenicity and chick mortality. Both laboratory and field data are available for these endpoints. The laboratory data is primarily available for mallard ducks (*Anas platyrhynchos*) and is limited to a relatively few number of selenium exposure concentrations in three separate studies. In contrast, the field teratogenicity data consists of relatively robust data sets ( $n = >100$  across a wide range of concentrations) for mallards, black-necked stilts (*Himantopus mexicanus*), and American avocets (*Recurvirostra americana*). The teratogenicity endpoint for the field data is very chemical-specific, with certain types of deformities readily associated with selenium poisoning. Field data for chick mortality is only available for black-necked stilts. Further, this endpoint is not selenium-specific and can be attributed to any number of factors including other contaminants, predators, weather, and starvation. Consequently, while the field data for teratogenicity provides a reliable data set for estimating effects thresholds, the field data for chick mortality is considerably more uncertain.

We evaluated the laboratory and field data by deriving dose-response relationships for the laboratory data using logit, probit and Weibull models of data pooled from the different studies. These dose-response relationships were then compared and contrasted to dose-response relationships for the field data synthesized by USFWS, which is the basis for their published thresholds.

## **RESULTS**

### **Fish**

Analysis of data pooled from several studies of bluegills (*Lepomis macrochirus*) and fathead minnows (*Pimephales promelas*) allowed us to derive dose-response relationships between larval mortality (the most sensitive endpoint) and selenium concentrations in ovaries, whole body, and diet for warmwater fish (Figures 1 through 3). From these dose-response relationships, we were able to estimate toxicity thresholds (expressed as the EC10) for these tissues. For warmwater fish, ovary, whole body and dietary thresholds of 17, 9 and 10 mg/kg dw were estimated.

For coldwater anadromous salmonids considerably less data is available. Several studies with rainbow trout (*Oncorhynchus mykiss*) are available, but the experimental designs only include water exposures, not water and dietary exposures, and so are not appropriate for deriving thresholds. Hamilton *et al.* (1990) is the only appropriate (i.e., includes exposure to dietary selenium) study for coldwater salmonids. In this study, Hamilton and colleagues evaluated the effects of waterborne and dietary selenium to larval chinook salmon (*Oncorhynchus tshawytscha*). The study was conducted for 90 days, but control mortality problems makes use of the Day-90 data questionable. Instead, use of the Day-60 data from the study appears more defensible (DeForest *et al.*, 1999). From these data, dose-response relationships for whole body and dietary tissue concentrations can be derived. The resulting thresholds (EC10) of 6 and 11 mg/kg dw indicate coldwater anadromous salmonids may be slightly more sensitive than warmwater fish (Figures 4 and 5).

## **Birds**

Similar to fish, by pooling laboratory data from several mallard duck studies, we were able to derive a dose-response relationship for duckling mortality and teratogenicity. Of the three models evaluated (probit, logit, and Weibull), we selected the most conservatively fitting model to derive a toxicity threshold (EC10) for each endpoint (Figures 6 and 7). The resulting toxicity thresholds are 16 and 26 mg/kg dw mean egg selenium for the chick mortality and teratogenicity endpoints, respectively.

## **DISCUSSION**

The USFWS has published a number of different thresholds for the various endpoints we evaluated in this study. In general, the USFWS thresholds are lower than those we estimated from the scientific literature (Table 1). The reasons differ for fish and birds.

**Table 1. Comparison of our recommended thresholds (mg/kg dw) to those of Lemly (1996) and Skorupa (1998).**

Tissue	Our Review	Lemly (1996)	Skorupa (1998)
Fish ovaries	17	10	N/A
Fish whole body	6 (coldwater anadromous fish) 9 (warmwater fish)	4	N/A
Fish diet	11 (coldwater anadromous fish) 10 (warmwater fish)	3	N/A
Bird Eggs – Teratogenicity	26	N/A	23
Bird Eggs – Chick Mortality	16	N/A	6

For fish, the exact basis for the thresholds advocated by Lemly (1996) is not entirely clear. The thresholds recommended are generally not supported by our review of the scientific literature, are inconsistent in many cases with the thresholds reported by the study authors, and also inconsistent with an independent interpretation of much of the same data recently conducted by USEPA scientists (Jarvinen *et al.*, 1999). In some cases, the tissue concentrations identified by USFWS as being associated with toxic effects are based on non-environmentally relevant exposure pathways, while in others the interpretations of the toxic tissue concentrations appear unsubstantiated by the data presented in the original studies. A study-by-study discussion of these inconsistencies can be found in DeForest *et al.* (1999).

For birds, the teratogenesis threshold we derived from laboratory data (26 mg/kg dw) is in reassuringly close agreement with the USFWS threshold derived from field data (23 mg/kg dw). In contrast, the chick mortality endpoint derived from laboratory data (16 mg/kg dw) differs significantly from the field-based threshold (6 mg/kg dw). That the field-based threshold is lower than the laboratory threshold is not surprising considering that the endpoint (chick mortality) is not selenium-specific. Consequently, in the field where other factors such as predators, weather and starvation are contributing to chick mortality, over-estimation of the selenium effects threshold is likely. Given that the experimental designs of the field studies have not been well documented in the peer reviewed literature, it is difficult to assess the validity of the field-based threshold (Fairbrother *et al.*, 2000). This threshold is put further into question by the close agreement between field and laboratory-based thresholds for teratogenicity, a selenium-specific endpoint. Given the similar thresholds for this endpoint, there is no reason to suspect the chick mortality endpoint would not be similar as well unless there were confounding factors associated with the field data. This appears to be the case.

## CONCLUSIONS

In general, we found the toxicity thresholds developed by USFWS to assess the environmental impacts of selenium are not well supported by the literature. After a careful review of available data, the basis of some of the thresholds advocated by USFWS remain unclear (e.g., fish ovaries and whole body). For other thresholds (e.g., bird eggs), the data may be confounded by other stressors resulting in overestimation of the threshold. We believe the alternative thresholds we have developed are more scientifically defensible and will provide a more accurate assessment of potential selenium impacts to the environment.

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